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Flach, Susanne; Maniam, Pavithran; Manickavasagam, Jaiganesh

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DR SUSANNE FLACH (Orcid ID : 0000-0001-6196-3706)

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E-cigarettes and head and neck cancers: A systematic review of the current literature

Corresponding author mail id: susanne.flach@googlemail.com

Susanne Flach[1,2], Pavithran Maniam[3], Jaiganesh Manickavasagam[1]

[1]Department of Otorhinolaryngology and Head & Neck Surgery and Tayside Medical Sciences Centre, Ninewells Hospital, Dundee, U.K.
[2]Department of Otolaryngology, Hospital of the Ludwig-Maximilians-University, Munich, Germany
[3]University of Dundee School of Medicine, Dundee, U.K.

Susanne Flach and Pavithran Maniam should be considered joint first author

Background
Cigarette smoking is a well established risk factor for head and neck (HN) cancers. Use of electronic cigarettes (e-cigarettes) is gaining popularity, being advertised as benign alternatives to tobacco. A wide variety of potentially harmful chemical components with variable quantity have been identified in e-liquids and aerosols of e-cigarettes. However, use of e-cigarettes remains controversial due to conflicting evidence.

Objectives
We aimed to assess the association between e-cigarettes and HN cancers. We conducted a systematic review to evaluate the literature for evidence on carcinogenic effects of e-cigarettes in the pathogenesis of HN cancers.

Type of review
Qualitative systematic review.

Search strategy
A Pubmed/Medline, Cochrane, CINAHL Plus, Trip medical database and Web of Science search was done for studies on e-cigarettes and HN cancer.

Evaluation method
Abstract review of all articles, full article revision of included studies, data extraction and quality assessment was performed by two independent assessors.

Results
The literature search resulted in the identification of 359 articles. Eighteen articles were selected for inclusion into the systematic review. The majority were laboratory-based studies, followed by several cohort and case studies, representing low-level evidence. A few reports suggested DNA-damage following exposure to e-cigarettes potentially due to increased oxidative stress. Flavoured e-liquids appear to be more harmful. There is variable evidence from clinical studies.

Conclusions
Our review outlines potential dangers associated with the use of e-cigarettes and their role in HN cancers. More longitudinal and controlled studies are needed to assess the possible link between e-cigarettes and HN cancers.

Keywords
e-cigarettes, electronic nicotine-delivery system, electronic cigarettes, smoking, head and neck cancer, head and neck neoplasm, oral cancer

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Conflict of interest
All authors declared that they had no conflicts of interest.

Introduction

Head and neck (HN) cancers are a heterogenous class of diseases, which comprises malignancies arising from mucosal surfaces in the oral cavity, pharynx, larynx, paranasal sinuses, as well as tumours originating from minor and major salivary glands.[1] The estimated numbers of new HN cancer cases and cancer deaths in Europe in 2018 were 161,200 (4.1% of all new cancer cases) and 72,800 (3.8% of all cancer deaths), respectively.[2]

Tobacco smoking remains a well established risk factor in the development of this type of cancer. Due to public health smoking cessation campaigns the incidence of HN cancers is slowly declining, with exception of Human Papilloma Virus-related cancers.[1] However, over the past decade electronic cigarettes (e-cigarettes) have entered the market, gained in popularity, and are now increasingly advertised as cheaper and benign alternatives to tobacco products because they lack the carcinogens from the latter.[3] According to results of the ASH Smokefree GB surveys on the use of e-cigarettes among adults in the U.K., in 2012 an estimated 700,000 adults used e-cigarettes. In 2017 this number quadrupled to 2.9 millions (6% of the U.K. adult population).[4] Proponents of e-cigarettes often advocate them as potential smoking cessation tools. A Cochrane systematic review reported evidence from two randomized controlled trials[5,6] that e-cigarettes containing nicotine help smokers to stop smoking in the long term (at six months or longer) compared with placebo e-cigarettes. No difference could be identified between the effect of e-cigarettes compared with nicotine patches.[7] A recent multi-centre, randomized, controlled trial of e-cigarettes versus nicotine-replacement therapy (NRT) showed that e-cigarettes were more effective for smoking cessation than NRT with accompanying behavioural

support. However, 80% of participants who used e-cigarettes and stopped smoking conventional cigarettes continued to use e-cigarettes at one year.[8] Contrary to these results, a systematic review by Kalkhoran *et al*.[9] combining observational and clinical studies came to the conclusion that e-cigarettes were associated with significantly less quitting among smokers, suggesting that further research is needed on this topic.

Also known as electronic nicotine-delivery system (ENDS) the modern e-cigarette was invented in China in 2003 and was introduced to the European market in 2006.[10] E-cigarettes are battery-operated devices that generate an inhalable aerosol by heating a solution (e-liquid) that contains a solvent (glycerin and/or propylene glycol), and various artificial flavours, with or without nicotine.[11,12] Currently there are hundreds of brands and models of ENDS; by 2014 more than 460 e-cigarette brands and more than 7000 e-liquid flavours were available in the U.S.A. alone.[10,13] To make it even more complicated, policies and legislations around e-cigarette product regulation, sale and advertisement vary widely world-wide. Until recently e-cigarettes were regulated in the U.K. as general consumer products without the need to report the content of e-liquids such as potentially harmful flavouring additives.[14] New restrictions and requirements were introduced to the U.K. with the Tobacco Products Directive 2014/14/EU from May 2016, including a minimum standard for safety and quality of e-cigarettes and e-liquids.[15]

Despite this legislation, the safety and potential long-term effects on health of e-cigarette use as substitute to tobacco products still remain controversial due to conflicting evidence in previous studies.[10,14,16-29] The role of e-cigarettes in HN cancer pathology in particular is therefore ambiguous and inconclusive to date. Our study is the first attempt to describe the association between e-cigarettes and HN cancers. This information may help otolaryngologists to communicate the risks and benefits of e-cigarettes to patients and to highlight health effects relating to diseases of the head and neck.

Therefore, the aim of this systematic review was to evaluate the effects of e-cigarettes on the pathogenesis of HN cancers as reported in the literature.

Methods

Ethical considerations

This study is a systematic review of previously published articles. No patient identifiable data was included.

A systematic search was conducted using MeSH terms and other relevant keywords in accordance to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement.[30] The final search was carried out on 18 September 2018. The search strategy is detailed in Appendix S1. Table 1 illustrates inclusion and exclusion criteria. The level of evidence was determined according to the guidelines published by the Oxford Centre for Evidence-based Medicine.[31] The work flow is illustrated in Figure 1.

Results

The initial search identified 359 potentially eligible articles. Fourty-four articles were provisionally selected and full reports of the relevant manuscripts were retrieved. These studies were supplemented by five additional articles identified by searching citation lists. Thirty-one articles that were initially included following abstract revision

failed to meet all the criteria and were excluded at the data extraction stage because they were not primary literature or there was no relevant data identified about e-cigarettes and HN cancers. Eighteen articles met our inclusion criteria (Table 1) and were included into the final qualitative analysis which was conducted as a narrative synthesis as shown in Table 2.

Evidence from basic research

Thirteen of the included studies were laboratory-based investigations. Nine of these articles used cells derived purely from the oral cavity[11,32-39] whereas one study worked with oropharyngeal cells and cells originated from a primary laryngeal tumour in addition to oral cells.[40] Cultures from the middle ear[41] and oropharynx[42] were also used to investigate the effect of e-cigarettes on these cells. Cytotoxicity of e-cigarettes in cellular experiments was demonstrated in eight studies[32-35,38,40-42], with varying extend of DNA damage[11,38,40,42] and oxidative stress induced by toxic components.[11,32,34] Ganapathy *et al*.[11] exposed cells to e-cigarette aerosol or tobacco smoke extracts. A nicotine-independent but dose-dependent increase in DNA damage induced by e-cigarette aerosols was observed, however, levels were lower than those induced by tobacco smoke extracts. Furthermore, the group showed that chronic exposure to e-cigarette aerosols could cause significant mutagenic oxidative DNA damage, with levels higher after long-term exposure to e-cigarette aerosol than to tobacco smoke, an increase in reactive oxygen species (ROS) and a reduced expression of proteins essential for DNA damage repair. In a similar study Yu *et al*.[40] investigated the effects of e-cigarette aerosols on a panel of cell lines. The *in vitro* study demonstrated an increase in DNA damage, arrest in G1 and G2, increased apoptosis, necrosis and cell death following exposure to e-cigarette aerosols. The same group published an abstract reporting significant induction of DNA double-strand breaks in cells incubated with e-cigarette aerosols as well as an increase in migration of HN cancer cells following e-cigarette treatment with upregulation of epithelial-mesenchymal transition (EMT)-promoting genes.[38] Increased cell migration in dysplastic oral keratinocytes following nicotine exposure was also demonstrated by another group.[39] Welz *et al*.[42] treated primary oropharyngeal mucosal cells with e-liquids and confirmed a significant reduction in cell viability as well as increased DNA damage following incubation with fruit flavoured e-liquids. Only one study used *in vivo* experiments to explore the reaction of e-cigarettes on the vocal cords of rats. Salturk *et al*.[43] detected hyperplasia and metaplasia of the laryngeal mucosa of rats following treatment with e-cigarette aerosols for four weeks, however, these results were not statistically significant. No chronic inflammatory changes were observed.

Evidence from the clinic

We identified just five clinical studies; two cohort studies[27,44], two case-control studies[45,46] and one case series[47]. The cohort study reported by Franco *et al*.[27] included 65 subjects who were divided into three groups (tobacco smokers, e-cigarette smokers, non-smokers) and were submitted to cytologic examination by scraping oral mucosa. The prevalence of micronuclei was significantly decreased in e-cigarette smokers compared to tobacco smokers and was similar to those of healthy controls. A pilot study investigating the effects of e-cigarettes on blood flow in the buccal mucosa in 10 subjects was reported by Reuther *et al*.[44] Here, an initial increase of capillary perfusion of the buccal mucosa was observed with nicotine-containing e-cigarettes. Bardellini *et al*.[45] enrolled outpatients for dental consultations

into two groups (former tobacco smokers, current e-cigarette smokers) and examining them for possible oral mucosal lesions (OMLs). The prevalence of OMLs was higher among e-cigarette smokers, however, the difference was not statistically significant. In terms of pre-cancerous OMLs, no difference was identified between the two groups. Bustamante *et al*.[46] analysed saliva from e-cigarette smokers, tobacco smokers and non-smokers, and demonstrated the endogenous formation of the oral and oesophageal carcinogen N'-nitrosonornicotine (NNN) in e-cigarette smokers. Finally, a case series reported by Nguyen *et al*.[47] described two cases of oral carcinoma associated with chronic e-cigarette use in otherwise healthy individuals. In both cases e-cigarettes were consumed 20 or 30 times per day for 13 years and a diagnosis of basaloid squamous cell cancer (SCC) in the oral cavity was made.

Discussion

Summary and discussion of key findings

There is some evidence suggesting a potentially carcinogenic role of e-cigarettes in the pathogenesis of HN cancers. Several studies commented on the cytotoxic effect of e-cigarettes. Korrapati *et al*.[38] showed that short-term treatment of normal epithelial cells with e-cigarette aerosols induced up to five-fold increase in cell death without nicotine and up to 10-fold increase with nicotine as compared to untreated controls. Similar results were demonstrated by Yu *et al*.[40], who, in addition to three other groups[11,37,42], confirmed the accumulation of DNA double-strand breaks in cell lines exposed to e-cigarettes. Various mechanisms have been suggested to be involved in this process, including the generation of high levels of 8-oxo-dG[11], a mutagenic DNA lesion, with increased presence of ROS[11,34,40], known to be linked to single- and double-strand breaks and oxidative DNA damage, as well as a decrease of total antioxidant capacity and reduced expression of DNA-excision repair proteins[11]. Interestingly, flavoured e-liquids, particularly menthol, seem to be more harmful compared to e-liquids without flavours.[36,37,41,42] Toxicants possibly responsible for these harmful effects have been identified in e-cigarettes. Particularly tobacco-specific nitrosamines (TSNAs), aldehydes, trace metals, volatile organic compounds, phenolic compounds, polycyclic aromatic hydrocarbons and tobacco alkaloids are potentially harmful and carcinogenic.[48-51] Exposure to high levels of formaldehyde has been reported to be associated with a lifetime cancer risk 15 times as high as the risk associated with long-term smoking.[52] In addition, it is linked to developing nasopharyngeal cancer, whereas TSNAs and nickel, one of the metals found in e-cigarette aerosol, have been implicated in oral carcinogenesis.[51,53]

Overall, it is difficult to directly compare different studies because the amount of toxic substances varies with e-cigarette brands, e-liquid flavours and solvents as well as device voltage. Some studies used e-cigarette aerosols[32,33,35,37,38,40,43], whereas others added e-liquids directly to the culture medium[34,36,41,42]. One would assume that exposure to aerosols would be more representative as fewer people would have direct mucosal contact with e-liquids. Most of the studies used cell lines as models which have the advantage of reproducability and easier maintenance compared to establishing primary cultures, however, it is often argued that cell lines in long-term culture may adapt to *in vitro* conditions and harbour genetic changes and adaptations required for their maintenance which differ from the original tissue. In a commentary to the article by Yu *et al*.[40], Holliday *et al*.[54] criticized the use of immortalized cell lines in addition to several methodological flaws, as well as the

failure to make the relevant comparisons to tobacco smoke. According to this commentary, the original results indicated that cells could survive longer in e-cigarette extracts compared to cells exposed to tobacco smoke extracts.[54] Nevertheless, the question whether toxicant levels in e-cigarettes are equivalent to those of carcinogenic substances in tobacco smoke remains debatable. Ganapathy *et al*.[11] and Welz *et al*.[42] reported that exposure to e-cigarettes induced less DNA damage than the equivalent exposure to tobacco smoke. However, in both cases, this conclusion was only drawn from comparison to previously published data and is therefore error-prone due to the variability of experimental conditions and set-ups.

Only very few studies have investigated e-cigarettes in context of HN cancers in the clinical environment. A case series by Nguyen *et al*.[47] described two patients with a positive history of chronic e-cigarette use who developed oral cancers, indicating a link between the long-term consumption of e-cigarettes and this type of cancer. Bustamante *et al*.[46] were able to identify a known carcinogen in the saliva of e-cigarette smokers. Even though the overall exposure to NNN was much higher in tobacco smokers, it is currently not known how much of a risk even smaller amounts of NNN pose to e-cigarette smokers, given the carcinogenic potency of NNN. In contrast to these results, a study by Franco *et al*.[27] could not identify a higher number of micronuclei, which are indicators of genomic instability, in e-cigarette smokers compared to non-smokers. Similarly, Bardellini *et al*.[45] found no differences in terms of pre-cancerous OMLs between e-cigarette smokers and former tobacco smokers. However, both studies had small sample sizes (65 subjects divided into three groups[27], 90 subjects divided into two groups[45]), limited exposure times (e-cigarette use for at least six months), used different e-cigarette devices with various types of e-liquids and device voltages.

Risk of biases, quality of evidence and limitations

Only a small body of literature was identified that was relevant to e-cigarettes and HN cancers. 75% of studies were applicable to oral carcinogenesis and only a minority of the reports looked into other HN cancers. This naturally created a bias towards oral cavity cancer in our literature search. In addition, the focus of the published literature so far has mainly been on SCC. Other malignant tumours such as adenocarcinoma, lymphoma or salivary gland tumours were not included. However, this also highlights the need for additional research into other HN cancers.

Most of the articles identified in our literature search, and described here, report basic laboratory experiments, either on established cell lines, primary cells or, even rarer, *in vivo* models, and so represent the lowest level of evidence. The remaining articles included poor-quality cohort studies with small sample sizes and limited exposure time, as well as case reports and case-series. They all suffered from limitations in their design such as the lack of proper control goups, selection bias and failure to account for confounding factors.

We excluded non-English primary articles from our initial literature search, which is why it might be possible that we have missed other relevant publications. Due to the lack of high-level evidence publications and the over-representation of laboratory-based research with a handful of case reports and poor-quality cohort studies, only low-level evidence could be included in this systematic review. Consequently, such findings are prone to biases from authors, to publication bias as well as ascertainment bias, and should be viewed with caution when it comes to the association between e-cigarettes and HN cancer pathogenesis.

Implications for research

Direct health effects of exposure to tobacco smoke have been extensively studied but e-cigarette research is still lagging behind comparatively considering the popularity with consumers.

Previous studies suggested a role for oxidative damage induced by e-cigarettes. Results, which need to be further investigated in order to establish whether this has an effect on the level of mutagenicity and hence the progression to cancer. Most authors rightly concluded that further research is needed to assess the effect of e-cigarettes on health. One step forward would be to move on from *in vitro* to *in vivo* studies and directly compare effects of tobacco smoke with e-cigarette aerosol on a histological, proteomic and genomic basis. In addition, more thoroughly designed prospective cohort studies over longer time periods and with larger sample sizes are necessary to establish the safety of e-cigarettes.

Implications for clinical practice

Although based on limited quality evidence, clinicians should act on the side of caution when advising patients about the use of e-cigarettes. The on-going popularity of e-cigarettes and the continued evolution in e-cigarette-like devices[55] requires a careful evaluation of the present evidence and honest discussions with patients about possible benefits and risks to consider.

E-cigarettes and other types of cancer

The effects of e-cigarettes are not limited to HN cancers and there is growing evidence to suggest that they may play active roles in the pathogenesis of other malignancies such as lung and bladder cancers.[56,57] For example, Lee *et al.* reported that e-cigarette aerosol exposure promotes DNA damage and impairs DNA repair in human lung and bladder cells, suggesting susceptibility of these cells to oncogenic transformation and carcinogenesis.[57] Other *in vitro* experiments using human airway epithelial cells and *in vivo* experiments on lungs of mice have demonstrated that aerosol from e-cigarettes induces oxidative stress, depletes glutathione and upregulates the production of inflammatory cytokines.[16,21] In addition, e-cigarette exposure has been shown to promote epithelial-to-mesenchymal transition in lung cancer cells, suggesting they may contribute towards metastasis in individuals at risk of lung cancer.[58] Measurement of bladder carcinogens in the urine of e-cigarette users demonstrated greater concentrations of carcinogenic aromatic amines, suggesting a potential role of e-cigarette in the pathogenesis of bladder cancer.[59] Current research on the role of e-cigarettes in cancer focuses primarily on HN, bladder and lung cancers and the majority of evidence in the literature is limited to either *in vitro* or *in vivo* studies. The safety profile of e-cigarettes in these cancers needs to be evaluated further using clinical studies to better understand the toxicological effects of e-cigarettes in promoting carcinogenesis.

Conclusion

The current literature on the association between e-cigarettes and HN cancer pathogenesis is poor. There is limited evidence that e-cigarettes are harmful and potentially carcinogenic for the head and neck, with some reports stating that e-cigarettes can lead to *in vitro* damage and that flavoured e-liquids are particularly damaging. There is currently no good quality evidence to conclude that e-cigarettes are less harmful than conventional cigarettes.

Keypoints

- This is the first systematic review to explore the association between e-cigarettes and HN cancer pathogenesis.
- We have synthesised data from 18 studies about HN cancers and e-cigarettes.
- Only low quality evidence from laboratory research, cohort and case studies was identified.
- There is evidence that e-cigarettes can cause *in vitro* damage, including increased DNA double-strand breaks and oxidative stress.
- E-cigarettes have only been on the European market for a bit longer than a decade and only recently evidence has appeared about their potentially harmful effects. Thus, not enough time has passed for long-term studies with larger cohorts and well-designed controls to emerge in the literature, or to draw a conclusion whether e-cigarettes play a relevant role in the pathogenesis of HN cancers.

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Table 1: Inclusion and exclusion criteria.

Criteria	Inclusion	Exclusion
Time period	No date restrictions	None
Language	English	Non-English
Age	Adolescents and adults	Children
Article type	All primary literature sources	Secondary literature
Study characteristics	Studies that included HN (oral, oropharynx, pharynx, larynx, nasopharynx) cancer/dysplasia/carcinoma in situ of any cell type and e-cigarettes.	Studies that did not include HN cancer/dysplasia/carcinoma in situ and e-cigarettes. Articles on smoking cessation. Study protocols. Guidelines. Articles about behaviour and beliefs.

Study	Country	Evidence type and level	Organ/tissue/cell type	E-cigarette type	Summary of author's conclusions
Song *et al.* (2018)[41]	South Korea	Bench research, level 5	Human middle ear epithelial cells (HMEECs)	E-liquids (12 brands)	Cytotoxic to cells; contain toxic heavy metals
Ganapathy *et al.* (2017)[11]	U.S.A.	Bench research, level 5	Human epithelial normal bronchial cells (Nuli1), human premalignant dysplastic oral mucosal keratinocyte cells (POE9n), human oral squamous cell carcinoma (UM-SCC-1)	5 e-cigarette aerosol extracts from 2 device types (NJoy, eGo-T)	Suppression of cellular antioxidant defenses; dose-dependent increase in DNA damage; potentially increased cancer risk
Ji *et al.* (2016)[32]	U.S.A.	Bench research, level 5	Normal human oral keratinocytes (NHOKs)	E-cigarette aerosol	Cytotoxic to cells due to oxidative stress induced by toxic substances
Welz *et al.* (2016)[42]	Germany	Bench research, level 5	Primary human oropharyngeal mucosal cells	3 e-liquids (2 fruit flavours, 1 tobacco flavour)	Cytotoxic to cells; DNA damage induction; potential risk factor for HN cancer
Salturk *et al.* (2015)[43]	Turkey	Bench research, level 5	Female Wistar albino rats (vocal cords)	E-cigarette aerosol (eGo-T)	No chronic inflammation; limited hyperplasia and metaplasia (not statistically significant)
Hwang *et al.* (2016)[33]	U.S.A.	Bench research, level 5	Human keratinocytes (HaCaTs)	E-cigarette aerosol (7 brands)	Cytotoxic to cells
Yu *et al.* (2016)[40]	U.S.A.	Bench research, level 5	Human keratinocytes (HaCaTs), human HN squamous cell carcinoma cells (HN30, UM-SCC-10B)	E-cigarette aerosol (V2, VaporFi)	Cytotoxic to cells; DNA strand break-inducing agent; potentially carcinogenic
Sancilio *et al.* (2015)[34]	Italy	Bench research, level 5	Primary human gingival fibroblasts	2 e-liquids (with and without nicotine)	Cytotoxic to cells; increased ROS production
Rouabhia *et al.*	Canada	Bench research,	Primary human gingival	EMOW e-cigarette	Altered cellular morphology,

(2016)[35]		level 5	epithelial cells	aerosol	cytotoxicity, increased apoptosis and lactate dehydrogenase (LDH) activity
Willershausen *et al.* (2014)[36]	Germany	Bench research, level 5	Human periodontal ligament fibroblasts	E-liquids (3 flavours)	Harmful effect of menthol additive
Sundar *et al.* (2016)[37]	U.S.A.	Bench research, level 5	Human periodontal ligament fibroblasts, human gingival epithelium progenitors, human gingival tissues (3D tissue model)	E-cigarette aerosol (BLU)	Increased oxidative/carbonyl stress, pro-inflammatory and pro-senescence responses associated with persistent DNA damage
Korrapati *et al*. (2016)[38]	U.S.A.	Bench research, level 5	Human epithelial and HN squamous cell carcinoma cells	E-cigarette aerosol (2 brands)	Cytotoxic to cells; DNA double-strand break induction; increased migration of HN cancer cells
Wisniewski *et al*. (2018)[39]	U.S.A.	Bench research, level 5	Human dysplastic oral keratinocytes (DOKs), human Leuk-1 cells, human spontaneously immortalized normal oral keratinocytes (NOK-SI)	Liquid nicotine	Increased cell migration by activating EGFR signalling through FASN-dependent mechanism; potential promoter of malignant progression in pre-cancerous lesions
Franco *et al.* (2016)[27]	Italy	Cohort study, level 4	Human oral mucosa scrapings	E-cigarette aerosol	Decreases prevalence of miconuclei compared to tobacco smokers; no harm caused in oral cavity
Reuther *et al.* (2016)[44]	U.K.	Cohort study, level 4	Human buccal mucosa	E-cigarette aerosol (with and without nicotine)	Increased capillary perfusion of buccal mucosa with nicotine-containing e-cigarette
Bardellini *et al.* (2018)[45]	Italy	Case-control study, level 4	Human oral mucosal lesions	E-cigarette aerosol	Linked to 3 types of inflammatory lesions in oral cavity (not statistically significant); no difference in terms of pre-cancerous lesions compared to tobacco smokers

Bustamante *et al*. (2018)[46]	U.S.A.	Case-control study, level 4	Human saliva	E-cigarette aerosol	Endogenous formation of carcinogenic N'-Nitrosonornicotine inside oral cavity
Nguyen *et al.* (2017)[47]	U.S.A./Vietnam	Case series, level 4	Oral carcinoma	E-cigarette aerosol	Development of oral cancer after chronic e-cigarette use

Table 2: Studies investigating the association between e-cigarettes and HN cancers.

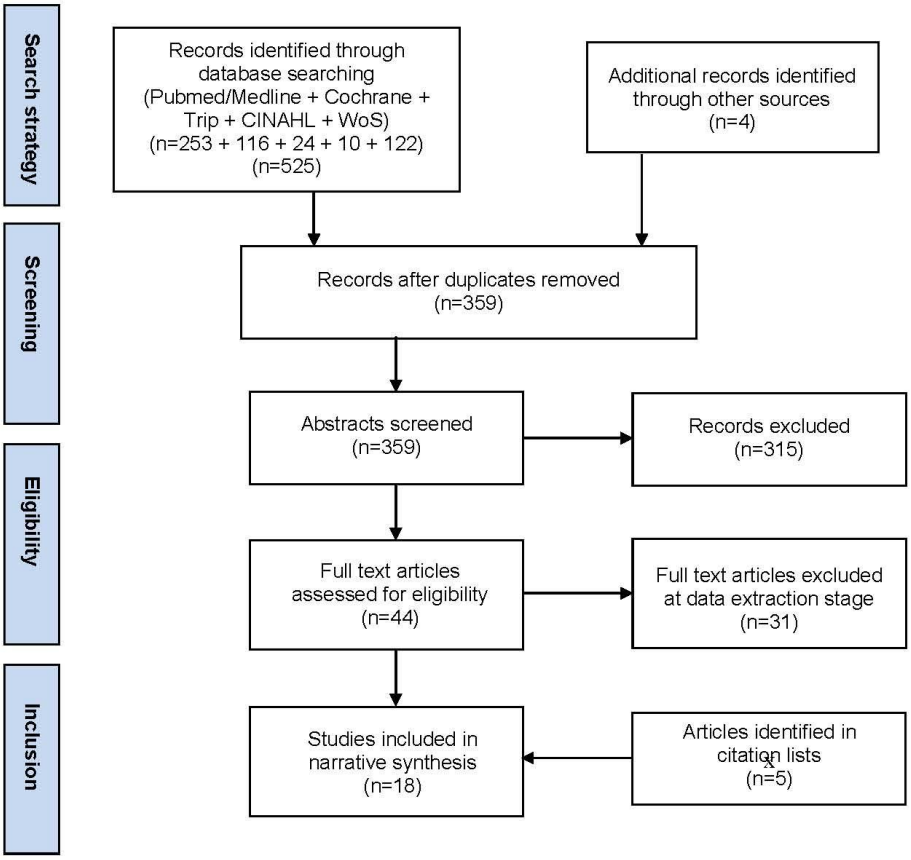

Figure 1: Flow diagram of the search strategy and screening process for final inclusion into the narrative synthesis.